Recovery and Purification

Controlling Process Performance and Product Quality

by Cheryl Scott

Modern separation and purification engineers are hard tasked with handling concentrated feed streams, recalcitrant proteins, and new contaminant profiles coming from the use of serum-free culture media. With regulatory, public, and industry attention increasingly focused on the subject of viral safety, for example, a risk-based approach following an FDA quality systems initiative continues to build momentum.

That regulatory perspective affects how recovery and purification unit operations are designed and conducted. Process modeling software is becoming increasingly sophisticated, so engineers can experiment in a virtual space before working with real (often expensive) chromatography skids and other hardware. Using new and improved analytical methods and scalable equipment, a downstream group can establish a "design space" for a given process. Automation is beginning to make inroads. And vendors offer options for addressing the downstream bottleneck — many of them based on single-use technology.

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Take-Away: Streamline downstream processing to improve cost and time to clinic, resolve bottlenecks, and ensure product quality.

Disposables and Disruptions

In a Friday morning session at the BioProcess International Conference and Exhibition in Providence, RI, Lynn Conley (associate director of process biochemistry in biopharmaceutical development at Biogen Idec) will describe how his company retrofitted a warehouse for manufacturing. The cell-culture suite includes two 1,000-L disposable bioreactors feeding two purification trains. This case study includes previously unpublished data. In the same session, Peter Rogge (director of downstream processing for Rentschler Biotechnologie) will provide another case study. He will focus on combining stainless steel and disposable virus filtration skids, with previously unpublished data from process and economical/analytical analyses.

Harvest and Recovery: Novasep is sponsoring a session on Thursday morning that highlights "disruptive" technologies for the first step in downstream processing. These include chromatographic approaches described by Ruby Leah Casareno of Seattle Genetics as well as centrifuges and depth filtration in case studies by Jason Condon of Janssen R&D, Thierry Ziegler of Sanofi, and Natraj Ram of Abbott's Bioresearch Center.

Ziegler asks whether centrifugation has any future in bioprocessing. He will present previously unpublished data from a project that replaced it with dynamic depth filtration. Ziegler calls it the method of choice for removal of cells and cell debris from high-density, fed-batch processes using mammalian cells. Depth filters are made of inert cellulosic materials that are often combined with active diatomite earth (DE). At 1,000-L scales and above, it usually has to be combined with precentrifugation, which adds cost and complexity. But Sanofi is working with Sartorius Stedim Biotech and ChangeXplorer to develop an alternative approach using Celpure pharma-grade DE. "This approach will use properties of DE that are well established in the food and plasma-fractionation industries," says Ziegler, "to improve filterability with the benefits of drastically reducing the surface area, process time, and capital expenditures required for harvest clarification." The final goal is a fully integrated, single-use system that will be scalable from milliliters to thousands of liters, replacing centrifugation in both laboratories and commercial operations.

Ram will also discuss the utility of novel depth-filtration media with

previously unpublished data. And Condon will present a case study evaluating single-use centrifugation technology in cell recovery. "Single-use technology is now commonly used for production of biological products," he says. "However, cell harvest is typically performed using nondisposable technology such as disc-stack centrifuges." His company applied a disposable fluidized-bed centrifuge to cell harvesting, first identifying and optimizing critical process parameters (CPPs) of the technology for clarification efficiency, which was determined by turbidity and particle-count measurements.

New chromatographic approaches show why such separations dominate bioprocessing. "Improvements in upstream processes result in high titers hindering downstream processing efficiency," Casareno points out. "SMCC is a bottleneck solution that streamlines purification efficiency." In her unpublished case study, significant benefits came from converting a high-titer MAb process from protein A batch processing to a sequential, four-column operation.

Viral Safety

Virus contamination presents such an important downstream challenge — especially to bioprocesses based on animal-cell production — that a special-focus track on Wednesday morning focuses exclusively on viral safety. Consultant and former EMA regulator Hannelore Willkommen will provide regulatory perspective on new trends and developments in the arena, and Millennium Pharmaceuticals' Norbert Schuelke will offer answers to questions regarding inconsistent results from contract testing laboratories.

Virus safety work is among the most commonly outsourced activities in biopharmaceutical development. Such testing involves highly specialized knowledge of viruses as well as intensely segregated laboratories. Dayue Chen of Eli Lilly presents an unpublished cospiking approach to demonstrating consistent and robust retrovirus removal with parvovirus filters. Mixed-mode chromatography offers another option, as suggested by Dan Bezila with previously unpublished data from Janssen R&D.

Genzyme's Kumar Dhanasekharan will describe a short-wave ultraviolet virus-inactivation device for cell culture media that was designed using computational modeling. "With high UV absorbances of cell culture media," he explains, "the primary challenge was to accomplish a sufficiently narrow UVC dose distribution at high flow rates to allow for media preparation in a reasonable time for a perfusion culture." His group experimentally verified their prototype using chemical actinometry techniques with fluorescent microspheres and media that respond to UVC by photobleaching. "Results indicate a viable device and technology for successful treating of cell culture media with high-throughput requirements for perfusion culture."

Jeri Ann Boose of Eurofins Lancaster Laboratories will step back for a look at the big picture through the lens of risk management. "Small, nonenveloped viruses represent a critical challenge to the biologics industry," she says. Recent contamination events involving vesivirus 2117 porcine circovirus (a calicivirus) and murine minute virus (MMV, a parvovirus) illustrate the problem. "The challenge," Boose elaborates, "is that these viruses are extremely resistant to physical and chemical inactivation." They can survive extremes of pH, high temperature, solvents and detergents, and gamma irradiation — methods that readily inactivate more fragile lipid-enveloped viruses. Boose will review the levels of clearance that can be achieved for these viruses with a range of techniques. She plans to highlight screening and treatment of raw materials, facility process flows, and cleaning validation studies as complementary strategies.

Achieving Better Process Understanding

The FDA's quality by design (QbD) initiative — and in particular its recently updated process validation guidance — requires biomanufacturers to demonstrate a level of process control that can only come from real understanding and intimate knowledge. At its core you'll find questions about the sources and extent of process variability. Another Friday morning session targets these efforts directly.

For chromatographic separations, addressing those questions begins with identifying variances and discovering their relationship with process performance — and ultimately product quality. Yaling Wu of Human Genome Sciences will present a few case studies demonstrating chromatogram variance in which process monitoring and follow-up investigations improved process understanding and provided for continuous improvement. "Chromatogram monitoring and trending has become a routine and powerful tool to ensure consistent performance of liquid chromatography columns and quality of the final product," says Wu. "Trending of chromatograms against a gold standard was implemented for both clinical and commercial manufacturing processes at HGS to ensure downstream purification processes are performing as expected."

For example, variability in the properties of chromatography adsorbents can lead to unacceptable process consistency. Ionela Iliescu (technical development scientist at Biogen Idec) will present a case study with previously unpublished data. "Column operating conditions may need to be designed to be adsorbent-lot–specific to achieve acceptable and consistent performance." A design-space strategy can help mitigate such risks, as in the anion-exchange chromatography step Iliescu will describe, by providing additional flexibility in column operating conditions for different chromatography adsorbent lots.

To reduce unanticipated sources of variability, downstream processes ideally should be optimized during scale-up, as Joseph Martin (senior scientist and research fellow in downstream operations at Pfizer)

points out. His company developed a three-step phase 1 monoclonal antibody (MAb) process using Sepharose Fast Flow anion and cation exchangers from GE Healthcare. Then the upstream group doubled the harvest titer in anticipation of market need. "Fixed tankage limitations in the intended commercial facility stimulated the use of high-capacity ion exchangers and a modification in the flow of the process streams," explains Martin, "which resulted in a doubling of the process throughput." New resins were then used in scaling up both the normal-titer and doubled-titer processes, yielding equivalent product quality and process performance in both versions.

In a related session on Wednesday, 10 October 2012, scaling down is the focus. High-throughput screening has found its way into downstream process development, as shown in presentations by John Welsh (senior research biochemical engineer at Merck), Paul McDonald (bioprocess development scientist at Genentech), and Lars Linden (senior purification and research analytic scientist in cell and protein science for Bayer Pharma).

"Several different microscale methods are available for high-throughput protein purification applications," says Welsh. Such methods can shorten development time using less material than traditional scale-down processes require. Using previously unpublished data, he will compare batch incubations, micropipette tips, and miniature columns, evaluating each in the context of platform purification adaptability and fermentation support. That can help identify the appropriate use for each technique. McDonald also offers a case study with previously unpublished data.

And Linden's presentation applies the miniaturization concept to assessments of "developability" for both antibodies and antibody–drug conjugates (ADCs). Together with cell-line productivity and cost-of-goods (CoG) analyses, he explains, these studies determine the manufacturing feasibility of a drug candidate. His company performs a thorough biochemical and biophysical characterization analysis along with downstream processing and analytics platform compatibility checks. This helps determine the intrinsic stability and technical robustness of a clinical candidate. It even includes early formulation buffer screening for accelerated formulation development.

A keynote address by Tony Cano (senior purification engineer at Genentech) on Friday morning will use a downstream purification case study with previously unpublished data to show the big QbD picture for a MAb biologic license application. He will bring together risk ranking and filtering tools, scale-down models, criticality assessments, and approaches to linking unit operations with overall design space.

Beyond Antibodies

Alongside the viral clearance specialty focus session on Wednesday morning will be a forum highlighting mixed-mode chromatography, hosted by Bio-Rad Laboratories. Most MAbs depend on protein-A affinity purification. Nonantibody therapeutics lack an equivalent affinity capture option, making them more difficult to purify. Mixed-mode media are playing an increasingly significant role in MAb polishing and are beginning to see application in the capture, intermediate, and polishing steps for other proteins.

Next-generation therapeutics — from proteins to cell therapies — are the sole focus of the last recovery and purification sessions on Friday afternoon, 12 October 2012. "The emergence of novel classes of antibody fragments creates new challenges for the development of standard downstream platforms," says André Dumetz of GlaxoSmithKline. He will present an approach that combines high-throughput and small-column process development for both early and late-stage process development, particularly emphasizing capture steps that can accommodate a range of molecular scaffolds.

Yiming Yang (senior purification process development scientist at Shire Human Genetic Therapies) applies a design-space approach to a 20 g/L nonantibody protein. "Protein charge profiles were identified as a critical quality attribute (CQA) that will affect protein solubility." Yang's group identified an ion-exchange (IEX) chromatography step as critical to protein solubility and performed a design of experiment (DoE) study to define a design space for controlling it. They began with a failure-mode and effects analysis (FMEA) risk assessment to rank process parameters in the IEX step, then screened high-risk parameters in a high-throughput platform using miniature columns from Atoll. That helped them identify the significant parameters that could affect solubility, recovery, and impurity clearance, which they could further study with scale-down column runs to define a design space. Finally, the group developed a process control strategy based on those results to better control both process performance and product quality.

Brian To of Bayer HealthCare focuses on PEGylated molecules in his presentation. "PEGylation has been shown to improve therapeutic protein half-life," he says. Efficiency of the process depends on concentrations of both the reductant and polyethylene glycol itself, as well as the incubation time for their reaction. His company optimized a reduction and PEGylation process to minimize by-products with a complex therapeutic protein. The scalable, integrated platform incorporates both processes into a single unit operation.

Other speakers will focus on specific recovery and purification technologies, from a novel affinity adsorbent to centrifuge and tangential-flow filtration approaches to cell therapies. Keith Selvitelli of Biogen Idec presents an unpublished case study involving factor VIII for hemophilia. "The development of a chromatography step that results in a product of high purity and yield is a major complexity," he says. Selvitelli will report on laboratory-scale process development and scale-up of GE Healthcare's VIIISelect affinity adsorbent.

Cell Therapies: Two presenters will discuss cell therapy processing. First,

Jacob Pattasseril of Lonza speaks of concentrating and purifying therapeutic cells. "As lot sizes increase to larger scales, it will be important to apply scalable bioprocessing concepts and technologies to therapeutic cell production," he says. "We have developed and characterized a scalable, closed-system concentration and purification technology for therapeutic-cell processing based on tangential-flow filtration (TFF)." Pattasseril will provide a case study using disposable and noninvasive sensor technologies to acquire real-time data for making process decisions, enhancing process knowledge, and monitoring process performance and product quality.

And finally, Paul Ko of Janssen R&D shares previously unpublished data on the use of fluidized-bed centrifuge (FBC) technology in cell therapy bioprocess development. His company used a FBC to process cell culture harvests by concentrating and washing cells for cell therapy applications. "In cell therapy bioprocess development," Ko says, "it is crucial to consider the capability to concentrate cells to an efficacious target dosage, dictated by viable-cell density, as well as reducing remnants of cell culture impurities such as serum to acceptable levels." By optimizing FBC process parameters such as flow rate and centrifugal force, his group demonstrated its effectiveness in separating cells from supernatant to achieve a targeted cell density dosage while removing undesirable impurities. "Overall cell loss during processing was minimal," Ko reports, "thus achieving a high cell yield from the FBC."

Streamlining Processes

As the "silo" mentality between upstream and downstream erodes, process engineers are increasingly looking for ways to streamline manufacturing to improve costs and shorten development times. A Thursday afternoon session will examine different means for reaching this end.

First, Stephen Hohwald (purification development engineer for Genentech) shows how ultrafiltration/diafiltration (UF/DF) can be used in manufacturing highly concentrated MAb formulations. "Monoclonal antibody therapeutics have traditionally been delivered using intravenous (IV) administration," he says. But "companies are now increasingly developing high-concentration formulations of MAb therapeutics to allow for subcutaneous (SC) administration. Howald explains how new UF/DF systems can be designed to minimize hold-up volumes and enhance recycle-tank mixing. But using legacy UF/DF systems can be challenging, as his case study will illustrate.

Next, process engineer Nikolas Brings of Biogen Idec presents an unpublished case study exploiting online refractometry for improved UF development and operation control. He echoes Howald's issues concerning highly concentrated drug products, especially as it relates to the use of traditional absorbance methods and process control techniques. Brings applied inline refractometry to measure concentration in real time for enhanced ultrafiltration process monitoring and control. He will provide data from both pilot- and clinical-scale manufacturing systems, where inline probes gave >96% concentration accuracy up to 150 g/L. The technology may have additional applications in bioprocessing.

Finally, Timothy Iskra will report on a previously unpublished study into the robustness and efficiency of a two-column purification process at his Pfizer biomanufacturing facility. And Yun Kang (head of ImClone Systems' purification team) discusses strategies for streamlining such platforms. "In two-column MAb purification platforms," he elaborates, "traditional Q columns — or increasingly, Q membrane adsorbers — are used as a polishing step in a product flow-through mode." But poor process performance can come with low-pI MAbs and those with solubility challenges under low–ionic-strength solution conditions. "We have developed a robust MAb purification platform," Kang says, "that demonstrates high process yield and efficient clearance of impurities (e.g., host-cell proteins and DNA, leached protein A, and high–molecular-weight molecules) for such challenging antibodies."

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Wednesday, 10 October 2012

8:00 AM – 12:00 PM	Special Focus: Mixed-Mode Chromatography Strategies for Biomolecule Purification (hosted by Bio-Rad Laboratories, Inc.)
8:00 AM – 12:00 PM	Special Focus: Viral Safety for Biologics
12:00–12:30 PM	Concurrent Technology Workshops
1:45–3:30 PM	Improving Predictability with Developability Assessments and Speed with High-Throughput Screening Methods

Thursday, 11 October 2012

8:00 AM –12:00 PM	Expanding the Tool Box: Disruptive Technologies in Harvest and Recovery (sponsored by Novasep)
12:00–12:30 PM	Concurrent Technology Workshops
1:40–3:45 PM	Streamlining Downstream Processing to Improve Cost and Time to Clinic

Friday, 12 October 2012

8:00–10:15 AM	Understanding Sources of Process Variability
10:15 AM – 12:15 PM	Impact of Disposable Technologies and Flexible Platforms and Manufacturing on Downstream Processing
1:25–3:00 PM	Developing Downstream Processes for Novel Molecules and Next-Generation Protein Therapeutics
3:30–5:00 PM	Developing Downstream Processes for Next-Generation and Novel Molecules

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Afterward, Thursday will close with a shared plenary session highlighting the potential for "truly continuous" bioprocessing. Konstantin Konstantinov (vice president of commercial cell culture development at Genzyme, a Sanofi company) will overview the necessary steps toward this goal of linking upstream and downstream operations and describe the advantages of doing so. Jens Vogel of Bayer Healthcare will offer some strategies for large-scale implementation of integrated continuous and semicontinuous downstream processing. Kenneth Green (operational excellence director at Pfizer) will "deconstruct" biomanufacturing while offering some previously unpublished data. "The complexity associated with biomanufacturing has increased over the past 30 years," he says. "Implementation of new technologies, quality risk management, improved process and product understanding allows the industry to rethink cost-effective ways to supply new products and markets." Emphasizing appropriate controls and facility design, he will describe how disposables can enable flexible and portable manufacturing with appropriate area classification to support concurrent multiproduct operations.

Cheryl Scott *is senior technical editor of* BioProcess International. *Quotes not otherwise attributed came from presentation abstracts.*

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